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AXIAL SUSCEPTIBILITY GRADIENTS IN ALGAE

C. M. CHILD

Introduction

Before presenting the data which constitute the subject of this paper, it is necessary to make clear the reasons which determined this venture into the botanical field and the presentation of these data to botanists for consideration and criticism. My experimental studies on the lower animals, extending over a period of years, have led me to certain conclusions concerning the nature of the physiological axes of organisms and of physiological individuality, which suggest that the physiological individual as an expression of a dynamic unity and order in protoplasm is fundamentally similar in its origin and nature in both animals and plants. The observations recorded in this paper constitute the first step in an attempt to determine whether and to what extent this is true. In order to make clear the point of view from which the investigation was undertaken, a brief statement of certain results of my experiments with animals and of the general conception toward which they point is essential.

I have shown that various lines of evidence point to the existence of a gradient in the rate of metabolism or of certain fundamental metabolic reactions as a characteristic feature of at least the chief physiological axes, and probably of all such axes in animals. Such a gradient is the earliest demonstrable indication of the axis, and in many forms persists without essential change throughout the life of the individual, while in others it undergoes modification during the course of development. In the axial metabolic gradient of the chief axis the region of highest rate of reaction becomes the apical region or head of the individual, and in other axes the development of organs shows a definite relation to the direction of the gradient.

In the final analysis such a gradient is not self-determined by some sort of organization, but arises as the result of the differential action of factors external to the protoplasm, cell, or cell mass acted upon. If, for example, an undifferentiated cell or cell mass is stimulated at some point by the action of a factor external to it, the resulting increase in metabolic activity is not limited to the region immediately affected, but a wave of change spreads or is transmitted over or through the protoplasm with decreasing energy, intensity, or physiological effectiveness, until, if the mass be large enough, it becomes inappreciable at a greater or less distance from the point of origin. It may be likened to a wave in water or in air spreading from the point of disturbance.

This is the simplest form of metabolic gradient, but while it produces temporary changes in the protoplasm, they are usually evanescent or the lasting effect is inappreciable. If, however, the stimulation be sufficiently often repeated or sufficiently long continued at the same point, more or less persistent changes in the protoplasm may occur, which appear as differences in its capacity to react. In such a case we say that the irritability of the protoplasm is altered. Since such changes are in general proportional to the energy or intensity of the transmitted change which produces them, they must differ at different distances from the point of action of the external factor, and the result is what we may call an irritability gradient, a gradient in the rapidity, capacity, or intensity of reaction of the protoplasm. Such a gradient is, I believe, the simplest form of persistent organic or physiological axis. In the chief gradient or axis, which is that first established, the region of highest metabolic rate or highest irritability becomes the apical region, and in the more highly differentiated animals the head, or more specifically the cephalic part of the central nervous system, develops from it.

In such a metabolic or irritability gradient it is evident that the region of highest rate of reaction must control or dominate other regions within a certain range, simply because the changes transmitted from it possess greater energy or physiological effectiveness than those from other regions, and therefore constitute the chief factor in determining the general metabolic rate in these other regions within the range of its action. In plants the dominance of the apical region over other levels of the axis is a familiar fact, and I have shown that in at least the simpler animals similar relations exist along the main axis, the region of highest metabolic rate being the dominant region.

This relation of dominance and subordination dependent upon a gradient in metabolic rate or in the irritability of the protoplasm is, as I believe, the simplest kind of physiological[1] unity and order in living protoplasm, these transmitted changes are the fundamental form of physiological correlation, and the physiological individual as an expression of dynamic unity and order consists fundamentally of one or more such metabolic gradients.

According to this conception, the individual originates as one or more quantitative gradients in the dynamic processes characteristic of the specific protoplasm concerned. Morphogenesis and differentiation result from the quantitative differences existing at different levels of the gradient because the substances which remain as constituents of or inclosures in the protoplasm differ not only in amount but in kind according to the rate or intensity of metabolic activity. In this way qualitative differences arise from quantitative, and what we call differentiation results. This conception of differentiation is supported by the fact that it is possible experimentally to alter widely the character of morphogenesis by means of conditions which act primarily in a quantitative way.

If this view be correct, the physiological axiate individual is fundamentally a unity resulting from transmitted dynamic changes,

[1] The word "physiological" is used here by way of distinction. Other kinds of individuality, of unity and order in aggregates, exist in the world and some of them undoubtedly exist in organisms, but by physiological unity and order is meant that kind which seems to be peculiarly characteristic of living things, and which determines orderly space and time relations with respect to an axis; in short, an orderly form and an orderly course of development.

rather than, as often assumed, from the transportation of specific chemical substances. In other words, physiological correlation is fundamentally transmissive rather than transportative in character, and transportative correlation arises only secondarily as the result of the quantitative and qualitative differences associated with the transmitted changes. It is evident that the existence of orderly transportative correlation demands the existence of differences of some sort arranged in an orderly way, and it is this fundamental order from which the other orderly phenomena in the life of the organism result, that this theory of the individual attempts to account for.

It is not necessary to assume, however, that every individual arises in the manner described above. When an axial gradient is once established in a cell or cell mass, it may persist through division or other forms of reproduction, so that each axis of the offspring is simply the axis of the parent or a fraction of it. In such cases the processes of rejuvenescence associated with reproduction bring the different fractions to essentially the same physiological condition, that is, the fraction which represents the lower levels of the parent gradient is brought up to the same metabolic rate or rates as that representing the higher levels. Nevertheless, we must, I believe, go back to a differential action of factors external to the protoplasm concerned for the origin of the metabolic gradient and so of the axis or polarity of which it is the simplest form.

Since the energy or effectiveness of a transmitted change in protoplasm decreases with increasing distance from the point of origin, the length of a metabolic gradient, and therefore the range of dominance of the region of highest rate, is limited, the limit varying with rate or intensity of metabolic activity, irritability and conductivity of the protoplasm, etc. This limit represents the maximum size which the physiological individual can attain and remain entirely an individual under the conditions which determine the limit. If, in consequence of continued growth, of decrease in the metabolic activity of the dominant apical region, of decrease in the conductivity of the protoplasm, any part previously within the range of dominance, that is, within the limit of length of the metabolic gradient, comes to lie beyond this limit, it becomes physiologically isolated, and if the irritability gradient in its protoplasm resulting from its former relations to the gradient persists, or if a new gradient arises in it, the reproduction of a new individual occurs. The space relations in the formation, inhibition, or development of buds in plants and the dominance of the chief growing tip seem to be fundamentally of this character. Growth in size of the plant or removal or inhibition of the chief growing tip results in physiological isolation of other parts, and new buds arise or buds previously inhibited develop.

This in brief is the conception of physiological individuality which has developed from my experimental work on the lower animals.[2] Since I have found metabolic gradients as characteristic features of the axes in animals and have been able to control and modify to a high degree size, development, and reproduction by controlling and modifying these gradients, it seemed desirable to determine whether the existence of similar gradients could be demonstrated in the simpler plants. The behavior in growth and development of the axis of the higher plant is a practical demonstration of the presence of a metabolic gradient with the apical region as the region of highest rate, but I thought it of interest to apply to some of the simple plants methods which I had used for the simple animals.

Methods and material

In the course of experimentation on the lower animals, I have found that certain relations exist between metabolic condition and susceptibility to at least many agents and conditions which interfere with, depress, or inhibit metabolism in some way. These relations are briefly as follows: To agents and conditions which affect the organism in sufficient degree to bring about death in a short time without permitting adaptation or acclimation, susceptibility varies directly with metabolic rate or with the rate of certain fundamental metabolic reactions, probably primarily the oxidations. The higher the rate of reaction the sooner death occurs, and vice versa. To agents and conditions which affect the organism to so slight a degree that more or less adaptation or acclimation is

[2] For more extended consideration of the subject see: CHILD, C. M., 1–4, 6, 7, 9–11 (chapter ix), 12.

possible, the rapidity and degree of acclimation varies under most conditions directly with the rate of metabolism; consequently in the long run the susceptibility varies inversely as the metabolic rate. Within certain limits under these conditions, the higher the metabolic rate the longer life continues, because the higher the rate, the more rapid and complete the acclimation. Thus with the same differences in metabolic rate, the relative susceptibilities to high concentrations of a given agent are the inverse of those to very low concentrations of the same agent.

These relations have been demonstrated for the cyanides, ethyl alcohol, ether and various other narcotics, acids and in marine forms for alkalies also, as well as for certain other substances and conditions, such as lack of oxygen, presence of carbon dioxide or other metabolic products in the medium, etc. It cannot be supposed that all these agents and conditions act on living protoplasm in the same way, but it is true that they all interfere with the activities of living protoplasm in some way. The general relation between susceptibility and metabolic rate is undoubtedly independent, at least to a large extent, of the particular method of action of a particular agent or condition. With a certain rate of chemical reaction in protoplasm, certain conditions as regards enzymes, aggregation of colloids, permeability, etc., are in general correlated. The chemical reactions may be retarded or inhibited by means of changes in these various protoplasmic conditions, as well as by a direct chemical action; and since all these different factors in the activity of the protoplasm are correlated, its susceptibility to at least many, if not all inhibiting agents and conditions the effect of which is not instantaneous but progressive, must vary in general with its metabolic activity. If the inhibiting effect is so great that the protoplasmic machine cannot adjust itself to the new conditions, the higher its rate of activity, the sooner it is brought to a standstill. But if the inhibiting effect is slight, the higher the rate, the more rapidly and completely adjustment occurs, because adjustment depends, at least in large part, on the activity of the protoplasmic machine, that is, upon metabolism.

These relations between susceptibility and metabolic rate have been demonstrated in many different ways, and within certain limits and with certain precautions can be used very widely as a means of comparing the general metabolic condition of different individuals, and of different regions of the body of a single individual. The method is so simple as to appear crude, but its delicacy and value have been shown very clearly. Practically it consists simply in finding the survival times of the different individuals or body regions to be compared in a concentration, determined by preliminary experiment, of the agents employed. I have found the cyanides particularly satisfactory for animals, because they are effective in very low concentrations and for various other reasons, but many other substances and conditions can be used in the same way.

In the two modifications of the method we have two ways of testing susceptibility and so of comparing general metabolic activity or fundamental features of it in different individuals and body regions. The first, in which susceptibility is directly determined by means of lethal concentrations, I have called the direct susceptibility method; the second, in which it is determined indirectly through the capacity for acclimation, I have called the indirect or acclimation method. The direct method is simpler and more satisfactory for most purposes, but can be controlled in many cases by the indirect.[3]

By means of these methods, checked and controlled by others, I have been able to demonstrate the existence of axial metabolic gradients as a characteristic feature of the animal organism, and various other lines of experiment indicate very clearly that the physiological axes are fundamentally metabolic gradients.

In my attempt to determine whether such axial gradients are present in plants the direct method was used, with the following procedure. The living plants, freshly collected, were first stained with the vital stain, neutral red, and then were placed in a solution of potassium cyanide in sea water. The neutral red was used as an indicator to make possible the determination of the time of death with some degree of exactness. In the plants thus stained and then killed in cyanide or various other agents of the proper

[3] These methods and some of their results have been considered in the following publications: CHILD, C. M., 4–10, 11 (particularly chapter iii), 12.

concentration, the first indication of approaching death is an increase in the redness of the stain, indicating an increase in acidity in the cell. After this the red color rapidly disappears, often within a few seconds, or changes to yellow and then disappears. In the more transparent cells the disintegration and coagulation and disintegration of the protoplast, sometimes preceded by a rather sudden plasmolysis, can be observed directly. In these cases the protoplasm, which up to this time has appeared under low magnification to be uniformly stained, suddenly becomes turbid and contracts into a small irregular mass in the cell or a number of such masses scattered over the cell wall. These masses contain all the neutral red as well as the phycoerythrin in cases where it is present, and in consequence of the increased acidity and the aggregation of the protoplasm, these masses usually appear blackish or purplish by transmitted light. Beyond question the moment of this change is approximately the moment of death. The appearance of the cell is completely altered in many cases. Instead of being uniformly red, it now appears nearly transparent, except in cases where the cell wall is also colored, and contains one or more blackish masses or granules. Following these changes the color rapidly disappears from the blackish masses of coagulated protoplasm, leaving the whole cell almost transparent and without color except in the wall. These changes are most readily followed in the transparent hairs or hairlike branches which occur on many of the Rhodophyceae, where they can often be observed with great clearness. It seems probable that at least in some cases the neutral red is decomposed with loss of color in the course of the death changes, but in other cases it becomes yellow before disappearing because the alkali of the KCN solution penetrates as the protoplast dies. These various changes, the deepening red color of the stain, followed by its disappearance with change to yellow in some cases, the visible disintegration and coagulation of the protoplast, and finally in the red algae the loss of the natural color by the extraction of the phycoerythrin are very definite and striking, and afford a means of determining with a considerable degree of exactness the time of death of different cells of the plant and so their relative susceptibility.

After testing various concentrations of KCN, I found that although different species showed considerable differences in susceptibility, a concentration of *m*/100 served the purpose for the forms examined, the survival time in this concentration ranging from a few minutes to several hours.

Neutral red itself, particularly in high concentrations, is somewhat toxic to living cells, and in the course of my experiments I found that a strong solution of neutral red in sea water could be used in the same way as cyanide to determine differential susceptibility, the only difference observed being that death occurs much less rapidly in the neutral red alone than in cyanide.

The work was done at the Marine Biological Laboratory at Woods Hole during August and September 1915. From among the forms available at that time, species with definitely axiate, and in most cases with more or less highly branched thallus and with a more or less definite growing tip (at least during the earlier stages of growth), were selected, because it was desired first of all to determine whether characteristic differences in susceptibility were associated with the visible axes of stems and branches. Most of the forms examined were Rhodophyceae, and it might be supposed that the natural color of these forms would interfere with the use of neutral red as an indicator. As a matter of fact, however, the color of the protoplasm stained with neutral red is distinctly different from the natural color, and there was no serious difficulty in observing the change and disappearance of color in any case, and the protoplasmic changes afforded additional means of determining the time of death in many cases.

For the identification of a number of the species I am indebted to Mr. GUSTAVSEN of the M.B.L. Supply Department. Others were identified with the aid of FARLOW'S *Marine Algae of New England*, supplemented by RABENHORST'S *Die Meeresalgen*. Thus far the growing thalli or parts of them have been examined in 14 species.

The susceptibility gradients

Enteromorpha sp.—Many plants of this species were examined, ranging from stems and branches in very early stages of development to large old thalli which had taken on the "intestinalis" characteristics. Young axes in good physiological condition always show a very definite susceptibility gradient from the apical end basally, that is, death with the disappearance of the neutral red begins at the tip of the stem or branch and proceeds basipetally with a high degree of regularity. The progress of the decoloration and death from cell to cell along the axis can be followed under the microscope. Occasionally a cell here and there or a small group of cells may die earlier or later than the other cells of their level, but in general the progress of death in the basal direction from the apical end is very regular in the younger axes.

In the large tubular axes, however, such a uniform death gradient does not appear. The first cells to die are scattered more or less over the whole length of the axis, or in many cases the death gradient is more or less distinctly acropetal in the more basal portions instead of basipetal as in the young axes.

These differences between the young and the large tubular axes are undoubtedly connected with the differences in the regions of growth and cell division in these different stages. In the newly formed developing axis where there is a more or less definite growing apical region, this is the region of greatest metabolic activity, and therefore a basipetal susceptibility gradient appears. Later, however, the activity of the apical region decreases and is apparently replaced by cell division and growth in the basal region or more or less along the whole axis. This change is associated perhaps with a greater or less degree of physiological isolation resulting from the decrease in activity of the apical region, which no longer dominates other parts to the same extent as earlier, and the earlier orderly behavior disappears. The original basipetal susceptibility gradient disappears, therefore, and is replaced by an acropetal gradient, at least in the basal region, or by a susceptibility mosaic. This method then makes possible an optical demonstration of the different localization of the highest metabolic activity at different stages. With a little care axes in all stages of this change can be found. In some the apical region is still active to some extent and a basipetal gradient extends for a short distance from it, while farther down the axis a susceptibility mosaic may appear, or the basal region may show a high susceptibility.

When the plant is killed by a high concentration of neutral red alone, the same axial differences in susceptibility appear as in death from KCN, although of course death begins later and progresses much less rapidly. The simple experiment of gradually killing the plants by overcrowding in standing water was also performed, and the changes in susceptibility and the progress of death were followed. After two days under these conditions, staining with neutral red showed that longer or shorter portions of the apical regions of the small short branches were dead and would no longer take the stain, while other parts still stained as living cells. In the large tubular thalli practically all cells were still alive at this time. After 5 days in standing water most of the short young branches were wholly dead, but in some a short basal region was still alive. The large thalli at this time showed a mosaic or patches of dead cells, often more marked in the basal region. These crude experiments are sufficient to show that differences in the susceptibility of different regions of the plant to overcrowding in standing water are essentially the same as to KCN. Death progresses in the same way in both cases.

One other point of interest in relation to the physiological age of parts may be mentioned. I have found it possible to use the susceptibility method very widely among animals to distinguish differences in physiological age, the young animals being more susceptible than the old because of their higher metabolic rate (11). In *Enteromorpha* the short branches, evidently in early stages of development, are more susceptible than the larger older branches. Where such short branches arise laterally on a larger stem, death proceeds basipetally in the short branch to the point where it joins the stem, but here it stops for the time being and the adjoining cells of the stem may remain alive for several hours after the branch is dead. The branch is evidently much younger than the stem at its level, and consists dynamically of a metabolic gradient of much higher rate than that of the stem, and this gradient ends rather sharply at its base. The gradient in the branch itself is also in a sense an age gradient, the cells of the apical region being youngest physiologically because of their continued reproduction, while basipetally the physiological age of the cells increases. In the large thalli where growth is no longer apical but more or less generally distributed, cells here and there have undergone more or less dedifferentiation, and so have become younger and resumed reproduction.

Where a thallus has been broken or injured, an area of high susceptibility extends for a greater or less distance, often 0.5 mm. or more from the region of injury. In the younger portions of the thallus where the basipetal gradient is still present, this area of high susceptibility arising from an injury extends farther in the basal than in the apical direction. Evidently this area represents the extent of the stimulation resulting from the injury, or at least the more persistent component of it.

The visible changes in the single cells as death approaches are essentially those described in the preceding section. First the color of the stain deepens, beginning in the young branches at the apical end and proceeding basipetally. Then as the protoplast undergoes disintegration and coagulation at death, the cell contents appear as an irregular blackish mass, but from this the color soon disappears and the dead decolorized cell is yellowish green instead of green under low powers, while under high powers the coagulated masses of protoplasm are visible.

The susceptibility method can undoubtedly be used for analyzing the metabolic conditions in *Enteromorpha* and related forms to a much greater extent than the few experiments described here indicate. As regards the question of metabolic gradients, however, these experiments give a definite answer. They show that the vegetative individual is dynamically in its earlier stages such a gradient, that each new branch is a new gradient and so a new individual, and that with advancing age and under certain environmental conditions the gradient may disappear more or less completely and give place to a mosaic of more active regions scattered over the thallus or more distinct basally.

Ectocarpus confervoides.—In this form the branches which are younger as regards development and the more apical regions of most others show a distinct basipetal gradient, while in the more basal regions of the more advanced parts of the thallus considerable irregularity appears, some cells dying earlier or later than in a regular basipetal gradient. These irregularities undoubtedly indicate the disappearance of the regular gradient in the later vegetative stages, with intercalary growth and cell division in the more basal regions. Here, as in *Enteromorpha*, elongation of the axis beyond a certain length, or decreasing activity of the apical region in consequence of advancing physiological age or for other reasons, may lead to a greater or less degree of physiological isolation of the more basal cells, which then again become active and therefore show a higher susceptibility.

Castagnea tuberculosa.—The complex structure and opacity of the thallus of this form interfered with the use of the susceptibility method, but it was found that the apical regions were most susceptible, and here in many cases the cells actually disintegrated in KCN as they do soon after death in many of the lower animals. Over most of the length of the thallus, however, no definite and constant gradient was observed.

The delicate transparent hairs which arise on the surface of the thallus afford interesting objects for susceptibility experiments. They consist of a single series of elongated cells and are unbranched. These hairs are very highly susceptible to KCN, and in *m*/100 they begin to die almost at once. Most hairs show in general a basipetal susceptibility gradient, but there are frequent irregularities, one or a few cells dying out of the order proper to such a gradient. Many if not all of these irregularities are undoubtedly due to injuries, for the hairs are so delicate that even the most careful handling of the plant is likely to break or bend them, but it may be that other factors are also concerned. The usual susceptibility gradient in these hairs indicates that their growth is primarily apical like that of most plant axes, but it is not improbable that in later stages secondary growth and division may occur in other cells as the activity of the apical region decreases.

Callithamnion (*roseum?*).—This monosiphonous form is very favorable for demonstration of axial differences in susceptibility. Both young plants consisting of a single stem with bipinnately arranged branches and more advanced stages in which branching had proceeded much farther were examined with similar results in all cases. In each branch death begins at the apex and proceeds basipetally from cell to cell. In each bipinnate system of stem and branches death begins at the apex of the stem and the most apical branches, and proceeds basipetally in the whole system, that is, the branches nearest the apex of the stem are most susceptible, those lower down are less susceptible, and so on to the base of the bipinnate system. The apex of the main stem of such a bipinnate system is highly susceptible like the apical branches, but the susceptibility decreases rapidly down the stem, so that at any level the stem is less susceptible than even the basal regions of branches at that level. These susceptibility gradients are remarkably regular and uniform with one characteristic exception. In the older more basal branches and in the stem of each bipinnate system of the thallus it was observed that the basal cell or the two most nearly basal cells were usually somewhat more susceptible and died earlier than the next two or three cells apical to them. In other words, the gradient in such branches is basipetal except for one or two cells at the base. The greater susceptibility of these basal cells suggests the possibility that they are the seat of secondary growth activity in consequence of physiological isolation, as in *Ectocarpus*, but whether this is actually the case or not I do not know. It may be merely that these cells are subjected to greater mechanical stimulation than more apical cells as the branches bend with water movements.

The greater susceptibility of more apical as compared with more basal branches in the same bipinnate system is undoubtedly due to the fact that the more basal branches are physiologically older and their rate of metabolism is lower than that of the more apical branches.

Pleonosporium Borreri.—Only very young plants of this species, consisting of a single unbranched axis of a stem with a few lateral branches, were examined. In every case a distinct basipetal susceptibility gradient was found, both in the main axis and in the lateral branches when they were present.

Ceramium rubrum and *C. tenuissimum*.—The susceptibility gradients in these two species are distinctly basipetal. The small cells of the extreme apical region are distinctly more susceptible than the larger differentiated cells below, but in the differentiated region the gradient is very regular for a distance of several millimeters from the apical ends when the plants are in good condition. In the more basal regions of the older, highly branched thalli, however, more or less irregularity usually appears, death occurring at the same time over lengths of 2 or 3 mm., or certain levels of the stem showing a higher or lower susceptibility than adjoining regions. Many very young unbranched individuals of *C. rubrum*, ranging from only 6–8 to 30–40 cells in length, which were found growing on other algae, were examined *in situ* and therefore in wholly intact condition. In these the basipetal susceptibility gradient was found to be very regular, and in the shorter individuals it extended to the base of the plant, while in some of the longer, some slight irregularity appeared in the basal region. As might be expected from the dichotomous branching in *Ceramium*, the susceptibility of all apical regions from the same general portion of a highly branched thallus was found to be about the same, except that in various cases where one member of a pair of young branches had been retarded in its development, the susceptibility of the retarded member was slightly less than that of the other. Different regions of the thallus, however, may show somewhat different susceptibilities even in the apical regions. For example, the lower peripheral portions of a large, highly branched thallus are often less susceptible than the upper parts, probably in consequence of different environmental conditions. *C. rubrum* is rather resistant to laboratory conditions as compared with other forms; the apical regions retain their susceptibility and the gradients persist with little or no change, even after a day or two in standing water.

The differences in susceptibility along the axis in *C. rubrum* are such that it requires several hours in KCN *m*/100 for death to progress from the growing tip to a level 3–4 mm. below it. In young unbranched plants about 20 cells in length, the progress of death over the whole length of the plant required about an hour in cases observed. In a few cases the progress of death from cell to cell was followed and timed for some distance. In the younger plants and the more apical regions of older axes the length of time between the death of one large cell of the axis and the next basal to it is only 2–3 minutes, while in more basal regions, where the metabolic rate is lower, it may be 15–20 minutes. The collapse and disintegration of the protoplast and the disappearance of color take place in a few seconds. In neutral red alone the axial differences in susceptibility are similar to those in KCN, but death begins much later and proceeds more slowly.

A few observations were made on the transparent hairs which arise from the cortical cells. These hairs contain no transverse cell walls, being merely cell outgrowths. In the long fullgrown hairs the susceptibility gradient is distinctly acropetal, while in those which are apparently still growing, it is basipetal. These differences suggest that while the hair is growing its apical region possesses the highest metabolic rate and the axial gradient is basipetal, but that after growth is completed and the apical activity decreases, the gradient becomes acropetal, that is, from the cell with which the hair is connected. The possible significance of this change will be considered later.

Spyridia filamentosa.—Observations on this form are fragmentary, since only a single thallus, detached and apparently not in perfect condition, was available for examination. Each main branch of the thallus, as well as each of the simple lateral branches which arise from it, shows in general a basipetal gradient; and each such system as a whole shows more or less clearly a basipetal gradient from branch to branch, the most apical branches being most susceptible, the next lower in general less so, and so on.

In the plant examined, however, irregularities in these gradients were frequent, both in single branches and in the whole system. Here and there cells were already dead, while others were more or less susceptible than their neighbors, and the same differences appeared between different branches in some cases. These irregularities probably indicate that the plant was not in good physiological condition; as previously noted, it was detached when collected and had been washed about by the waves, so that some of the cells had undoubtedly been injured or killed. The susceptibility of this species to KCN and to neutral red is in general very high, death beginning almost at once in KCN $m/100$, and within half an hour to an hour in neutral red alone. Since I have found that high susceptibility to the cyanides in animals indicates in general high susceptibility to at least many other depressing conditions, I am inclined to regard this species as rather sensitive and readily injured physiologically, and such a high susceptibility would readily account for the irregularities observed in the death gradient.

Champia (*Chylocladia*) *parvula*.—Various stages of development, ranging from unbranched to large much branched thalli, were examined. In general, death begins at the apical end of each axis. A short terminal portion, evidently representing a growing tip, dies first, and then, after a more or less distinct interval, death proceeds slowly basipetally. In the younger, shorter axes the gradient is usually very regular over most of the length, and in the older axes over the apical 2–3 mm. Farther basally, however, irregularities appear in cells or cell groups. Whether these irregularities indicate physiological isolation and secondary growth in these basal regions, or whether they are the result of injuries or other external factors, I do not know. They are certainly more frequent in the longer, older axes than in the shorter, younger ones. In general also the younger axes, or at least their more basal regions, show a higher susceptibility than the older axes, so that while the death of the growing tip may occur at about the same time in both, the basal region of the younger branch may die considerably earlier than that of the older branch.

The transparent, unicellular hairs which develop on the thallus usually show an acropetal susceptibility gradient in the cases examined, like the hairs of *Ceramium rubrum*, although in some hairs the gradient is basipetal and in some death begins at both ends and proceeds toward the middle. Further observations are necessary to determine with certainty the meaning of these differences, but it seems probable that, as in *Ceramium*, the growing hair may have a basipetal gradient, the full grown hair an acropetal gradient, and that those in which death begins at both ends are stages in the change from one condition to the other. It may be, however, that stimulation or injury is responsible for these differences in some cases at least.

Chondriopsis (*Chondria*) *dasyphylla* var. *sedifolia*.—In young unbranched or slightly branched plants in good condition and usually in the more apical regions of the branches in older much branched thalli, the susceptibility gradient is basipetal. Irregularities in the course of death and acropetal gradients observed in some cases probably result from injury or bad environmental conditions which affect the more active regions to a greater extent than the less active regions, or they may indicate perhaps the completion of apical growth in an axis. In general the physiological ages of different branches on a single axis are indicated by their susceptibility, the smaller less advanced branches being the more susceptible.

In some of the plants examined cystocarps were present. In the sterile wall of the cystocarp the gradient is basipetal, as in the vegetative portions of the plant. Differences in susceptibility in the cystocarp contents, indicating differences in physiological age, were also observed. No attempt was made to distinguish the various stages in the development of the carpogonium and carpospores, but it was merely noted that the susceptibility of the fully developed carpospores was much less than that of the cystocarp contents in the earlier stages. This difference indicates that the carpospore has the low metabolic rate characteristic of physiologically old cells.

The transparent hairs which arise in tufts at the apical ends of the thallus branches are beautiful objects for the observation of the susceptibility gradient. Each hair is monosiphonous and dichotomously branched, and the susceptibility gradient in each hair as a whole is very distinctly and uniformly basipetal, the apical cell of each branch dying first and death progressing basipetally from cell to cell in regular order.

In each single cell of the hair, however, the susceptibility gradient is almost invariably acropetal. The disintegration of the protoplast and the appearance of the dark masses resulting from coagulation begin at the basal end and proceed very uniformly to the apical end of the cell, the progress of death over the whole length of the cell often requiring 2–3 minutes. With rare exceptions the death of each cell is completed before that of the next cell basal to it begins.

My observations were limited to hairs which were apparently full grown or nearly so, and only further work can determine whether young actively growing hairs will show the two opposed gradients or not. Discussion of the facts is postponed to the general section of the paper.

Polysiphonia variegata and *P. fibrillosa*.—In *P. variegata* the general axial gradient is clearly basipetal in each axis, with a few irregularities in freshly collected plants. The transparent, dichotomously branching hairs also show a basipetal gradient, both in the hair as a whole and in the single cells, so far as observed. After a day or two in the laboratory irregularities in the susceptibility are much more frequent.

Cystocarps in various stages of development were present on some of the plants examined, as in *Chondriopsis*. The cystocarp wall shows a basipetal gradient. In general the susceptibility of the cystocarp, except in the earliest stages of its development, is lower than that of the cells of the vegetative axis from which it arises, and it was further noted that the region of the branch to which the cystocarp is attached also shows a lower susceptibility than adjoining regions of the axis. This low susceptibility may extend basipetally over 4 or 5 segments of the thallus, and acropetally over 2 or 3, and is more marked in connection with more advanced than with earlier cystocarps. Apparently the cystocarp in its later stages, together with a portion of the vegetative thallus in the region of its attachment, represents a region of lower metabolic activity than the adjoining vegetative regions.

As in *Chondriopsis*, the cystocarp contents in the earlier stages are much more susceptible than the fully developed carpospores which are the least susceptible portions of the whole plant on which they occur. Apparently metabolic activity in the carpospores is relatively slight.

P. fibrillosa is in general much more susceptible then *P. variegata*, and after a few hours in the laboratory the course of death along the axes is very irregular. In freshly collected plants, however, a basipetal gradient usually appears clearly in the more apical regions, but even here the irregularities are very frequent a few millimeters from the tips, and sometimes death occurs first at some level below the tip. I think it probable that these irregularities result from stimulation or injury of the apparently very sensitive cells in collecting and handling. The great difference in susceptibility between the two species is evident from the fact that in *P. fibrillosa* death begins almost at once and is usually completed within a half hour or less in KCN *m*/100, while in *P. variegata* even the more apical regions live in the same concentration from half an hour to an hour, and the less susceptible parts die only after several hours.

Dasya elegans.—In both the polysiphonous axes of the thallus and the very numerous monosiphonous, dichotomously branched secondary branches, the susceptibility gradient is in general distinctly basipetal. In the secondary branches, however, irregularities appear, chiefly near the base. As in *Callithamnion*, the basal cell or the two most basal cells of the older branches very often die before those above them. The suggestion made in the case of *Callithamnion* may apply here also, namely, that these cells are more stimulated mechanically than those above them by the movements of the branch with the water currents. It may be, however, that some degree of physiological isolation of the basal region has occurred in the older branches.

A basipetal gradient also appears in the system of secondary branches on an axis as a whole, the most apical branches being most susceptible, those lower down less so, and so on. This is true even for the apical regions of the secondary branches, and indicates their differences in physiological age, corresponding in general to their level on the axis, the most apical being the youngest, the most basal being the oldest.

The elongated stichidia present on some of the plants examined also show a basipetal susceptibility gradient, and the gradient in development of the tetrasporangia in the stichidia is paralleled by a susceptibility gradient, the most basal and so most advanced tetraspores being the least susceptible parts of the plant on which they occur.

Discussion

The observations on these 13 species indicate, so far as they go, that in each definite vegetative axis of these forms, at least in plants that are in good condition and not too old, a more or less definite susceptibility gradient exists. To these may be appended some fragmentary observations on the blue-green alga *Calothrix*, which indicate that even in this simple form a basipetal susceptibility gradient exists. Although the observations are incomplete at many points and further work on other stages and species is necessary, the uniformity of results is striking and cannot but suggest that the order observable along these axes and the susceptibility gradients are in some way associated. If the susceptibility method is of any value as an indicator of general metabolic or oxidative activity, the susceptibility gradient represents a gradient in metabolic or oxidative activity, the most susceptible regions being the most active. If this be true, the axes of these simple plants possess primarily the same metabolic characteristics as the axes of animals in this respect. Such a fundamental resemblance or rather identity as this must possess some fundamental significance, and in the introductory section of this paper my views concerning this significance are briefly stated. Many lines of evidence have forced me to the conclusion that this axial metabolic gradient is the basis, the starting point of the order in space and time which is characteristic of the axes of organisms. In short, according to this point of view, the axis, whatever else it may become during development, is fundamentally a metabolic gradient. The order along the axis is primarily an expression of this gradient. The organic or physiological individual, as distinguished from other kinds of individuality which may exist in the organism and in the inorganic world, consists fundamentally of one or more metabolic gradients. The various aspects of this conception of the organic individual and their bearing on biological problems have been considered elsewhere (12), and it is impossible here to do more than note the complete agreement with the general conception of these observations on plants.

From this point of view there is a fundamental identity in the apical regions of plant and animal organisms and in their relations to other parts. I have suggested elsewhere (12, pp. 189–192) that the fact that the apical region of the animal gives rise to the highly specialized and physiologically stable cephalic nervous system or brain, while that of the plant remains indefinitely or for a long time more or less completely embryonic, must be connected with certain differences in the metabolic processes in the two groups. Morphological structure in the animal is to a large extent primarily colloid and protoplasmic, while in the plant the structural substances are mainly carbohydrates, and the protoplasm itself shows little permanent or persistent structure. Apparently in plant metabolism the proteids and other protoplasmic substances produced are less stable under physiological conditions than in the animal, and so do not persist as structure but are continually undergoing change and reconstitution, particularly in regions of high metabolic activity. In the animal, on the other hand, some relatively stable molecules arise even in regions of the highest activity; consequently morphological structure appears, even in these regions, and such structure is more stable than structure developed in regions of lower metabolic rate. Because of these differences the apical region of the animal undergoes differentiation, and its relations to other parts determine that this differentiation shall take the form of a transmitting system, the nervous system, which brings about a high degree of physiological integration, while in the plant the apical region usually remains embryonic or differentiates very slowly. Transmission exists only in relatively primitive form and the degree of physiological individuation in the plant remains low.

In the unicellular hairs of *Ceramium* and *Champia* the susceptibility gradient is usually acropetal in the full grown hairs, but apparently basipetal in the growing hairs and in the multicellular branching hairs of *Chondriopsis* the gradient of the hair as a whole is basipetal while that of single cells is acropetal in the full-grown hairs. In the multicellular hairs of *Castagnea* the gradient is usually basipetal, but a distinct intracellular gradient could not be distinguished. Further work is necessary for positive conclusions, but a suggestion is perhaps permissible. These hairs of course are incapable of photosynthesis and are dependent, therefore, on other parts of the plant for nutrition. The course of nutritive substances must therefore be acropetal in direction in them. In view of the facts available, it seems probable that during the growth of the hair the apical region has the highest metabolic rate and the gradient is basipetal. Under these conditions each level lives, so to speak, at the expense of the level or levels basal to it. At this stage the gradient is probably basipetal throughout, at least in some cases; but as senescence progresses and the activity of the apical region decreases, the nutritive factor may become more important in determining the metabolic rate at different levels. Since the direction of movement of nutritive substance must be acropetal in each cell, the basal portions of the cell are most favorably situated and perhaps therefore show a higher metabolic activity with an acropetal gradient, provided relations to more apical cells do not obliterate or reverse it. In this way a gradient in the cell opposite in direction to the gradient in the hair as a whole may arise, at least in the later stages of development. It may be that in some of the multicellular hairs this condition is present from the early stages of development, but the fact that in the unicellular hairs of *Ceramium* and *Champia* the gradient is basipetal in what appear to be the earlier stages of development, and acropetal in the fully developed hairs, indicates that such change may occur. It remains, however, for future investigation to determine whether these suggestions are correct.

Attention has been called incidentally to the differences in susceptibility which apparently indicate differences in physiological age, such, for example, as the lower susceptibility of fully developed as compared with small developing axes, and the low susceptibility of the carpospores. The basipetal axial gradient itself is in a sense an age gradient, for the physiological age of the cells increases from apex to base, the apical cell or cells remaining physiologically young because of continued division and cell reproduction, and the other cells differentiating and growing old more or less rapidly according as they divide less or more frequently. We may say in fact that the axial gradient is a more or less definite gradient in cell behavior, and it is this which must be accounted for. It is here, as I believe, that the physiological dominance of the apical region plays a rôle. This region is the first part of the axis to appear, and it originates as a region of high metabolic activity, either in consequence of physiological isolation from other apical regions or by the local action of external factors. After it has once arisen it becomes, as I have endeavored to show, the chief factor in determining the metabolic rate in the other regions of the axis to which it gives rise, so far as they are within the range of its influence. Since the changes transmitted from it to other regions undergo a decrement in energy or effectiveness in the course of transmission, a more or less definite gradient in metabolic activity and therefore in behavior of the cells results. The more active cells divide more frequently and differentiate and grow old less rapidly than the less active cells. The apical region itself, as the most active region of all, may remain embryonic and physiologically young indefinitely if the processes of senescence and rejuvenescence balance each other in it; but if this is not the case it may gradually grow old, though senescence is less rapid in it than in other parts because of the higher metabolic rate. According to this conception, therefore, an apical region arises wherever the metabolic activity of a cell or a cell group, or in unicellular forms of a part of the cell protoplasm, becomes high enough to enable it to dominate or control to some degree the metabolic activity of other cells within a certain range. When the apical region has arisen, the development of the plant or animal individual proceeds as the specific constitution of the protoplasm and the conditions determine.

As soon as differences between different parts along the gradient arise, chemical correlation, that is, the influence of substances produced in one part upon another, must begin to play a part, and this is unquestionably a factor of great importance in determining the further course of events along the axis. It is evident, however, that chemical correlation cannot occur in an orderly way unless orderly differences already exist at different points or levels, and I have endeavored to show how these differences may find their origin in the axial metabolic or irritability gradient (12). If my conclusions are correct, the physiological axes in both animals and plants consist in their simplest form of such a gradient, and it is the interrelation or correlation in this gradient which determines the orderly behavior in relation to this axis.

Summary

1. In 14 species of marine algae, including 10 species of Rhodophyceae, 2 of Phaeophyceae, 1 of Chlorophyceae, and 1 of Cyanophyceae, a more or less definite gradient in susceptibility to KCN exists in the vegetative axes, and in several species in other parts also; the susceptibility being greatest, at least during growth and development, in the apical region and decreasing basipetally.

2. Since extended experiment with the lower animals indicates that the degree of susceptibility to cyanides and to many other agents and conditions is in a general way, and within certain limits, a rough measure of metabolic activity or of certain fundamental metabolic processes, probably primarily the oxidations, these axial differences in susceptibility in the algae are regarded as indicating the existence of axial metabolic gradients. If this conclusion be correct, the axis in these simple plants is identical with the axis of the animal organism in this respect.

3. The relations of physiological dominance and subordination, of physiological isolation and of differentiation to metabolic gradients, are briefly considered.

HULL ZOOLOGICAL LABORATORY
UNIVERSITY OF CHICAGO

LITERATURE CITED

1. CHILD, C. M., Studies on the dynamics of morphogenesis and inheritance in experimental reproduction. II. Physiological dominance of anterior over posterior regions in the regulation of *Planaria dorotocephala*. Jour. Exper. Zool. 11:187–220. *figs. 21*. 1911.
2. ———, *ibid.* III. The formation of new zooids in *Planaria* and other forms. Jour. Exper. Zool. 11:221–280. *figs. 36*. 1916.
3. ———, *ibid.* IV. Certain dynamic factors in the regulation of *Planaria dorotocephala* in relation to the axial gradient. Jour. Exper. Zool. 13:103–152. *figs. 46*. 1912.
4. ———, *ibid.* V. The relation between resistance to depressing agents and rate of reaction in *Planaria dorotocephala* and its value as a method of investigation. Jour. Exper. Zool. 14:153–206. *figs. 2*. 1913.
5. ———, *ibid.* VI. The nature of the axial gradients in *Planaria* and their relation to antero-posterior dominance, polarity, and symmetry. Arch. f. Entwickelungsmech. 37:108–158. *figs. 13*. 1913.

6. CHILD, C. M., Certain dynamic factors in experimental reproduction and their significance for the problems of reproduction and development. Arch. f. Entwickelungsmech. **35**:598–641. *figs. 3.* 1913.

7. ———, The axial gradient in ciliate infusoria. Biol. Bull. **26**:36–54. *figs. 25.* 1914.

8. ———, Starvation, rejuvenescence, and acclimation in *Planaria dorotocephala*. Arch. f. Entwickelungsmech. **38**:418–446. *figs. 3.* 1914.

9. ———, A dynamic conception of the organic individual. Proc. Nat. Acad. Sci. **1**:164–172. 1915.

10. ———, Axial gradients in the early development of the starfish. Amer. Jour. Physiol. **37**:203–219. *figs. 10.* 1915.

11. ———, Senescence and rejuvenescence. Chicago. 1915.

12. ———, Individuality in organisms. Chicago. 1915.